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The Distribution of Total and Organic Mercury in Seven Tissues of the Pacific Blue Marlin, *Makaira nigricans*[1]

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ABSTRACT: Tissue samples from Pacific blue marlin (*Makaira nigricans*) were collected at a fishing tournament in Kona, Hawaii, in August 1973. Analyses of total and organic (methyl) mercury indicated that the marlin may be biotransforming methyl mercury to inorganic mercury such that about 90 percent of the body burden of mercury is in the inorganic form. Specific analysis of a subsample of the tissues showed that the difference between the total and organic mercury concentrations was equal to inorganic mercury by weight.

IN THE LAST DECADE, numerous papers have reported mercury concentrations in various species of fish throughout the world. Studies done on museum specimens revealed that mercury levels in fish currently being sampled were similar to those levels in older, preserved fish, and that mercury contamination may be from natural rather than human causes (Barber, Vijayakumar, and Cross 1972; Miller et al. 1972). Although there has recently been some question of the accuracy of testing museum specimens (Gibbs, Jarosewich, and Windom 1974), it would seem likely that many aquatic organisms have been exposed to geological fluxes of mercury throughout much if not all of their evolutionary history.

Rivers, Pearson, and Shultz (1972), while carrying out a survey of the mercury concentrations in nine species of fish from Hawaiian waters, reported that the mercury in eight of the species was predominantly organic mercury, a finding consistent with others in the literature (Westöö 1967; Kamps, Carr, and Miller 1972). In Pacific blue marlin captured in August 1971, it was found that the organic fraction comprised less than 20 percent of the total mercury in the muscle, and less than 7 percent in the liver. In a study of marlin sampled in August 1972, this disparity between the levels of organic and total mercury was again found (Shultz et al. 1976). Dr. Gunnel Westöö (private communication) has identified the organic fraction as methyl mercury. In these previous studies, no attempt was made to show that the difference between total and methyl was inorganic mercury.

For this report, samples of seven different tissues from 35 Pacific blue marlin were analyzed for total, organic, and inorganic mercury. In addition, samples of muscle tissue were baked in a vacuum oven to permit us to ascertain if any loss of mercury occurred.

MATERIALS AND METHODS

Samples of muscles, liver, spleen, gonad, stomach, gill, and blood were collected from 35 fish landed during a billfish tournament in Kona, Hawaii. The tissues were ground with Dry Ice in a Waring blender. The organic extraction was carried out as described by Rivers, Pearson, and Shultz (1972), i.e., a benzene extraction of the mercury compound was reextracted with cysteine, then oxidized with permanganate, and analyzed by the flameless atomic absorption apparatus. Total mercury digestions were performed as described by Rivers, Pearson, and Shultz (1972), except that the 30 ml of concentrated nitric acid was reduced to 10 ml. The inorganic method involved a nitric acid digestion at ambient

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TABLE 1

TOTAL AND ORGANIC MERCURY IN SEVEN TISSUES OF 35 PACIFIC BLUE MARLIN

FISH WEIGHT (kg)	SEX	MUSCLE		LIVER		SPLEEN		GILL		GONAD		STOMACH		BLOOD	
		TOTAL	ORGANIC	TOTAL	ORGANIC	TOTAL	ORGANIC	TOTAL	ORGANIC	TOTAL	ORGANIC	TOTAL	ORGANIC	TOTAL	ORGANIC
		(mg/kg, wet weight)													
49	F	0.13	0.06	0.22	0.03	0.07	0.07	0.05	0.04	0.05	0.04	0.08	0.03	–	–
60	M	0.76	0.26	2.20	0.15	1.72	0.10	0.16	0.10	0.11	0.07	0.42	0.05	0.13	0.03
64	M	1.13	0.10	2.78	0.01	1.66	0.08	0.10	0.07	0.12	0.04	0.30	0.04	0.08	0.01
68	M	2.42	0.15	8.69	0.05	5.08	0.08	0.24	0.04	0.33	0.05	0.06	0.07	0.30	0.01
68	M	1.26	0.34	2.48	0.26	1.84	0.16	0.22	0.12	0.19	0.09	0.43	0.23	0.08	0.04
71	M	2.74	0.31	17.40	0.11	5.28	0.09	0.33	0.07	0.30	0.07	1.03	0.12	0.09	0.03
71	M	2.72	0.36	7.59	0.10	4.28	0.10	0.17	0.08	0.29	0.08	0.82	0.12	0.13	0.03
75	M	6.80	0.51	30.80	0.26	10.40	0.11	0.34	0.14	1.60	0.19	1.56	0.10	0.16	0.02
75	M	1.54	0.07	6.00	0.05	3.16	0.05	0.23	0.06	0.13	0.08	0.48	0.04	0.25	0.01
77	M	1.76	0.36	4.70	0.14	3.64	0.09	0.23	0.08	0.26	0.11	0.52	0.10	0.12	0.02
78	M	1.68	0.51	5.60	0.18	2.56	0.10	0.20	0.05	0.25	0.11	0.48	0.14	0.06	0.02
79	M	1.74	0.25	6.30	0.15	2.82	0.07	0.15	0.06	0.30	0.08	0.82	0.12	0.12	0.03
79	M	3.30	0.25	9.00	0.06	3.88	0.08	0.27	0.07	0.43	0.11	1.12	0.10	0.40	0.03
83	M	3.30	0.29	13.00	0.09	6.60	0.07	0.34	0.08	0.48	0.10	1.08	0.12	0.77	0.03
86	M	4.32	0.46	18.20	0.04	5.48	0.12	0.37	0.10	0.56	0.13	1.16	0.12	0.52	0.05
86	M	3.92	0.50	20.00	0.04	14.40	0.12	0.31	0.07	0.51	0.17	1.36	0.09	0.12	0.06
87	M	3.88	0.46	13.00	0.15	11.40	0.15	0.23	0.05	0.56	0.12	1.46	0.16	0.10	0.05
90	M	6.76	0.21	38.80	0.11	23.80	0.08	0.44	0.10	0.94	0.08	2.60	0.06	0.28	0.05
91	M	3.32	0.43	14.40	0.19	7.20	0.13	0.31	0.10	0.39	0.10	1.34	0.13	0.07	0.05
97	M	2.20	0.64	4.36	0.27	3.04	0.25	0.25	0.19	–	–	–	–	0.86	–
97	M	3.12	0.43	9.80	0.15	6.50	0.15	0.31	0.09	0.46	0.13	0.95	0.20	0.41	0.03
102	F	2.48	0.44	5.30	0.13	2.48	0.21	0.22	0.21	–	–	–	–	0.09	0.05
102	M	4.48	0.44	32.80	0.15	15.00	0.21	0.45	0.14	0.44	0.15	1.48	0.13	0.44	0.05
109	F	0.70	0.19	1.08	0.11	0.54	0.15	0.11	0.11	0.16	0.11	0.26	0.10	0.14	0.03
113	F	5.39	0.69	13.60	0.22	–	–	0.34	0.19	0.50	0.18	1.20	0.14	0.38	0.05
139	F	2.68	0.69	10.00	0.26	5.64	0.30	0.45	0.19	0.74	0.26	–	–	–	–
139	F	4.76	0.46	9.20	0.30	8.20	0.15	0.33	0.13	0.31	0.11	1.50	0.20	–	–
144	F	2.68	0.82	3.30	0.33	4.40	0.35	0.31	0.26	–	–	–	–	0.22	0.19
144	F	4.76	0.48	4.80	0.48	6.30	0.30	0.28	0.13	0.30	0.11	0.95	0.29	0.24	0.17
145	F	13.30	0.47	27.00	0.30	25.00	0.33	0.46	0.30	2.78	0.30	2.06	0.33	0.28	0.17
177	F	4.12	0.95	6.80	0.41	–	–	0.48	0.31	2.70	0.83	–	–	0.15	0.15
181	F	16.80	0.97	58.80	0.43	–	–	0.86	0.26	2.46	0.40	–	–	0.68	0.09
189	F	9.10	0.71	24.00	0.57	14.00	0.30	0.64	0.15	2.38	0.31	1.92	0.33	–	–
244	F	15.60	0.77	28.40	0.38	21.00	0.38	0.91	0.12	–	–	–	–	0.94	0.18
415	F	6.09	0.75	10.20	0.56	–	–	0.49	0.18	1.84	0.33	1.38	0.39	0.53	0.21
Average Values		4.35	0.45	13.45	0.21	7.33	0.16	0.33	0.13	0.74	0.16	1.06	0.14	0.29	0.06
Methyl Hg/ Total Hg (%)			10.4		1.5		2.2		38.6		22.0		13.6		21.9

NOTE: mg/kg wet weight are for all tissues. Mercury has been calculated as inorganic mercury.

temperature as described by Iverson and Hierliky (1974). All analyses were made with a Perkin-Elmer 303 atomic absorption spectrophotometer equipped with a Manning vapor chamber (Manning 1970) or a Coleman mercury analyzer. No difference in results was detected between these two instruments.

Analyses of a large number of samples on a single day pointed to problems in the determination of mercury by the flameless absorption technique that have received little attention in the literature. The continued use of a glass reaction chamber for the reduction of mercury to its elemental state results in the formation of a nearly imperceptible and highly insoluble coating of an unidentified residue on the glass. Consequently, the standard solutions in a reduced state cannot be left standing in these reaction vessels without a steady loss of mercury. This problem can be avoided by maintaining an excess of potassium permanganate in the solutions until immediately before beginning analysis. Gage and Warren (1970) noted the formation of an insoluble stannous film on the reaction chamber while carrying out low-level mercury analyses of urine specimens and observed that after a few determinations the analyses could proceed without further addition of stannous chloride. The authors suggested that this was due to the same insoluble film, presumably composed of stannous oxide with some residual reducing properties. They noted that the film could be removed by dilute nitric acid, although it has been our experience that only prolonged soaking in concentrated dichromate cleaning solution will remove this film. Another problem involved the use of the Coleman mercury analyzer. After running 30 to 40 samples, we had to change or wash thoroughly the tubing and cell to avoid erratic readings. A stannous film coating was also observed on the biochemical oxygen demand bottles and they too had to be rigorously cleaned quite frequently. When samples were run only once a week, however, flushing of the cell and tubing proved unnecessary.

A cooking experiment was carried out to determine if the mercury in the muscle could be reduced by baking the muscle in a vacuum oven for 1 hour. Samples of fillet were ground with Dry Ice and analyzed before and after being cooked. The temperature of the oven ranged from 55° to 93° C at 20 in. of mercury vacuum.

RESULTS

Total and organic mercury concentrations for muscle, liver, spleen, gill, gonad, stomach, and blood are given in Table 1. The lowest total mercury levels were found in blood (average 0.29 ppm) and highest in liver (13.45 ppm). The lowest organic mercury concentrations were found in blood (0.06 ppm) and the highest in muscle (0.45 ppm). The percentage of organic to total mercury ranged from 1.5 percent in liver to 38.6 percent in gill. In muscle tissue, methyl mercury comprised about 10 percent of the total mercury.

Inorganic mercury analyses were performed on a subsample of muscle, liver, and spleen (Table 2). The inorganic mercury results equalled the difference between the total and organic values within the limits of experimental error. This confirms the earlier assumption that the preponderance of mercury in the marlin is in the inorganic form.

In all tissues except liver and stomach, total mercury levels showed a dependence on weight and organic mercury levels. Organic levels were highly correlated with weight in all tissues (Table 3). Figure 1 shows this relationship of mercury to weight in the muscle tissue. An analysis of variance indicated no significant difference between mercury levels in males and females. All but one female weighed over 100 kg and all but one male weighed under 100 kg.

The results of the cooking experiment are given in Table 4. No loss of mercury was observed after 1 hour of baking.

DISCUSSION

Mercury reaching the marine environment could be passed to the fish directly from the water and/or indirectly via the food chain. Both pathways may be active but their relative importance is not known. Voyce and Zietlin (1974) reported mercury concentrations from nearshore and offshore stations in Hawaiian waters and found levels higher in the open

TABLE 2

INORGANIC MERCURY ANALYSES

FISH TISSUE AND WEIGHT OF FISH (kg)	INORGANIC MERCURY SPECIFIC DETERMINATION* (mg/kg)	INORGANIC MERCURY BY DIFFERENCE TOTAL Hg/ ORGANIC Hg (mg/kg)
Muscle		
49	0.11	0.07
64	0.78	1.03
68	0.89	0.92
75	0.66	1.47
79	2.90	3.05
86	4.18	3.86
97	2.90	2.69
102	1.81	2.04
102	3.92	2.69
113	4.98	4.70
Average	2.31	2.25
Liver		
68	9.19	8.64
71	18.08	17.29
75	6.39	5.29
75	29.60	30.54
78	4.44	5.42
79	8.22	8.94
83	11.09	12.91
87	13.18	12.85
97	4.44	4.09
102	5.82	5.17
Average	11.05	11.18
Spleen		
71	4.98	5.19
71	4.31	4.18
75	8.38	10.29
77	4.05	3.55
87	9.86	11.25
90	23.66	23.72
144	4.05	4.05
Average	8.47	8.89

* Specific inorganic mercury method from Iverson and Hierliky (1974).

ocean samples. The mercury was found to be entirely inorganic in nature and showed decreasing concentrations with increasing depth, suggestive of atmospheric input. Concentrations from 0 to 200 meters depth ranged from 109 to 224 ng/liter at an open ocean station off the island of Oahu. In a study of Hawaiian coastal waters, Lau (1973) found that mercury values averaged less than 20 ng/liter. Other mercury values reported for seawater samples ranged from 0.5 to 364 ng/liter (Olafsson 1974), with an estimated worldwide average of about 100 ng/liter (Miller et al. 1972). If direct absorption from water were the primary route into the fish, the gills could be expected to contain a very large fraction of inorganic mercury. Conversely, the percentage of methyl mercury (39 percent) was greatest in this tissue.

It would appear that diet is a more important mercury source to the marlin. Stomach contents examined by the National Marine Fisheries Service on 76 marlin captured in 1973 showed that the most common food items were tuna (38-percent occurrence), mackerel scad (36 percent), squid (21 percent), and spiny puffer (19 percent) (Naughton 1973). Although mercury values are not available for all of the food items, Rivers, Pearson, and Shultz (1972) have reported concentrations in two species of tuna caught off of Hawaii: yellowfin (*Neothunnas macropterus*) and skipjack (*Katsuwonus pelamis*), with mercury levels of 0.54 ppm and 0.38 ppm, respectively. Another food item of the marlin is the dolphin, *Coryphaena hippurus* (12-percent occurrence in stomach contents), which was found to contain 0.25 ppm mercury. These fish are pelagic species and, as such, are exposed to the same amount of mercury in their physical environment; yet, all have mercury levels nearly an order of magnitude less than that of the marlin. In addition, their mercury content was essentially all organic mercury, i.e., methyl mercury.

If most of the mercury entered the marlin via the food chain as methyl mercury it would seem that demethylation to inorganic mercury was occurring. The high proportions of inorganic mercury in the liver and spleen suggest that these organs may be locations for this biotransformation, although this does not mean that they are necessarily the sole or even primary sites of reaction. If the conversion is catalyzed by a specific enzyme system, then the liver and spleen would be prime areas. However, the change may be the result of a chemical reaction of the organomercurial with thiol groups of cysteine, glutathione, proteins, and/or other molecules as suggested by Weiner et al. (cited by Norseth and Clarkson 1970). Under such conditions the release of inorganic mercury could be expected wherever methyl mercury

TABLE 3

Correlation Coefficients of Weight versus Mercury Concentrations in Various Marlin Tissues

tissue	total Hg/weight	organic Hg/weight	total Hg/organic Hg
Muscle	0.53** (35)	0.65** (35)	0.57** (35)
Liver	0.23 (35)	0.79** (35)	0.27 (35)
Spleen	0.54** (31)	0.86** (31)	0.41* (31)
Gill	0.64** (35)	0.50** (35)	0.49** (35)
Gonad	0.61** (31)	0.57** (31)	0.82** (31)
Stomach	0.34 (28)	0.77** (28)	0.40* (28)
Blood	0.43* (31)	0.81** (30)	0.36* (30)

Note: Numerals in parentheses indicate sample numbers.
* $P < 0.05$.
** $P < 0.01$.

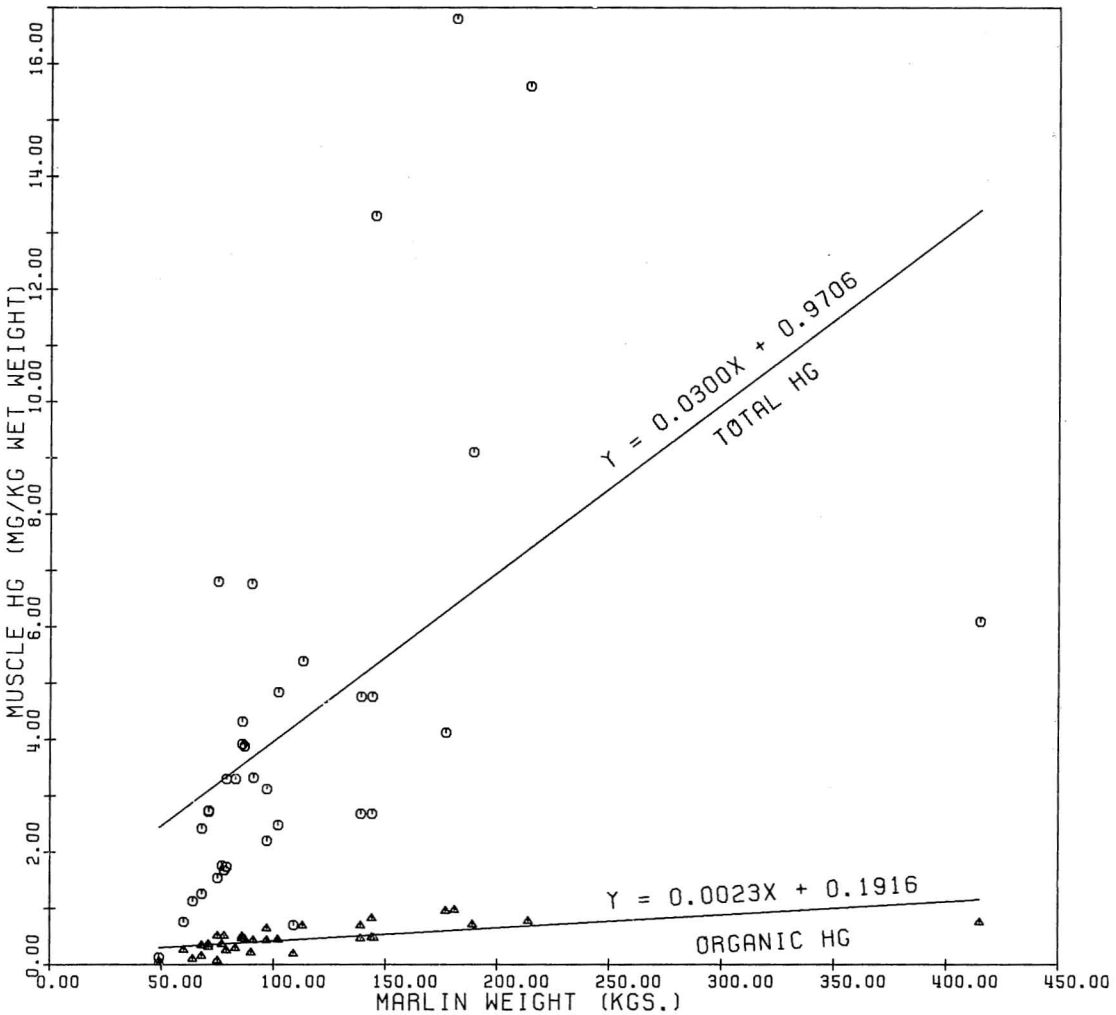

Figure 1. Regression curves describing the relationship of total and organic mercury in muscle tissue of the Pacific blue marlin to fish weight. Circles, total mercury; triangles, organic mercury.

TABLE 4

RESULTS OF COOKING EXPERIMENT

FISH SAMPLE WEIGHT (kg)	COOKED VALUE mg/kg	UNCOOKED VALUE mg/kg
86	3.92	3.92
87	3.76	3.88
90	6.70	6.76
91	3.52	3.32
97	2.36	2.20
97	3.36	3.12
102	2.28	2.48
102	4.90	4.84
109	0.71	0.70
113	5.00	5.39
Average	3.65	3.66

NOTE: Samples were cooked in a vacuum oven for 60 minutes, 20 in. Hg, at 55° to 93° C.

compounds occur in the body (Norseth and Clarkson 1970). Since inorganic mercury and methyl mercury are both widely distributed in the marlin tissues, this explanation seems plausible. Conversion of methyl mercury to inorganic mercury has been shown to occur in another species of fish, the bluegill (Burrows and Krenkel 1973) and in rats (Norseth and Clarkson 1970).

Using the data for marlin sampled in 1971 and 1972 (Rivers, Pearson, and Shultz 1972; Shultz et al. 1976) and the data reported here, we carried out an analysis of variance between sexes and between years. No significant difference ($P > 0.05$) was found between males and females for either total or methyl mercury. Comparison of samples from 1971, 1972, 1973 indicated no difference in total mercury ($P > 0.05$) but the methyl mercury values showed highly significant differences ($P < 0.01$). The difference with the organic mercury may be due to sampling variability resulting in a high adjusted mean of 0.899 for 1971 versus 0.430 and 0.434 for the other 2 years. We performed these statistical analyses using the muscle tissue as representative of the whole fish, since this tissue represents the bulk of the fish weight and stores most of the mercury in the organism. It appears then that the mercury concentration of the marlin as a statistical group has remained approximately constant over the past 3 years. The mercury levels of the individual fish, however, have not remained constant as the levels have increased with size (age). Since the marlin are open-ocean carnivores, it is possible that these mercury tissue levels have existed for extended periods.

It is unlikely that the mercury in the marlin is due to man's input, since direct contamination with industrial and/or agricultural effluents is nearly impossible with a pelagic species. Hammond (1971) estimated that man's industrial and agricultural inputs of mercury into the ocean basins since 1900 are two to three orders of magnitude less than the 10^8 metric tons of mercury considered to be present in the oceans. Except for restricted coastal areas, it does not appear likely that man has increased the mercury content of the oceans by even as much as 1 percent. Natural global inputs such as volcanic activity may play a large role in supplying pelagic fish with mercury. Additions of mercury to the atmosphere in the Hawaiian archipelago from volcanic sources have been shown to be substantial (Eshleman, Siegel, and Siegel 1971), and relatively high amounts of mercury have been found in plants and fungi near an active volcano on the island of Hawaii—near the area where the marlin in this study were caught (Siegel et al. 1973).

Since marlin is eaten by segments of Hawaii's population and since the average total mercury value is greater than the federal guideline of 0.5 ppm, we decided to cook the meat to see if any mercury loss occurred. Westöö (1966) has reported that boiling did not remove methyl mercury from fish tissue, and Rohala et al. (1973) have shown that baking at 200° C does not remove inorganic mercury as $^{203}Hg(NO_3)_2$ from spiked liver paste. Table 4 shows that no mercury, either organic or inorganic, was lost from marlin muscle after it had been baked in a vacuum oven for 1 hour. The mercury concentration of the raw muscle, then, is representative of the mercury in cooked meat.

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